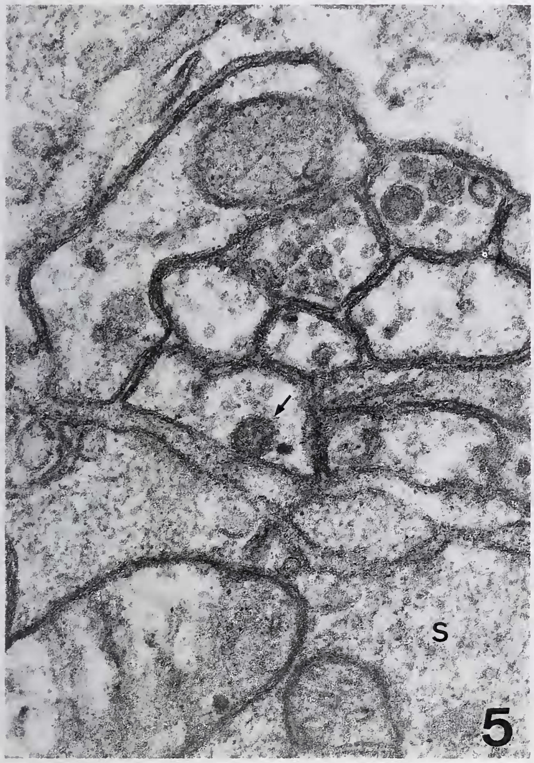


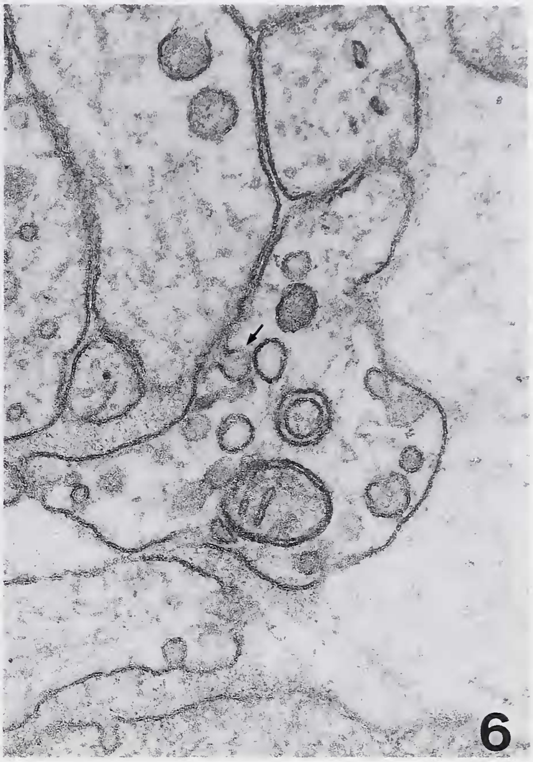


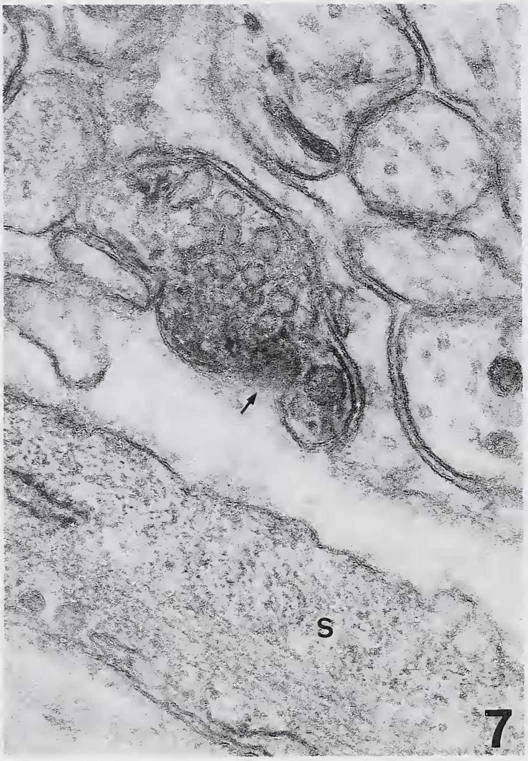
FIG. 5. Exocytotic figure of large cored vesicle (arrow) at the surface of nerve fascicle, distant from smooth muscle cell (S) in the circular muscle coat. ×67,000.

FIG. 6. Exocytotic figure of large cored vesicle (arrow) at the site facing other neuronal process, in the circular muscle coat. ×61,000.

vesicles in the neuromuscular junctions is known to occur at the conventional synaptic sites [12]. In the present study, I found the exocytotic figures of small clear vesicles not only in the vicinity of smooth muscle cells but also in the sites distant from smooth muscle cells. This difference may be caused by the absence of apparent synaptic contact between the enteric neurons and smooth muscle cells.

In the central nervous system of the rabbits, Nitsch and Rinne [13] observed the exocytotic figures of large cored vesicles at the synaptic junctions of the hippocampal mossy fibers. However, recent electron microscopic studies [1, 2] demenstrated the presence of non-synaptic release of large cored vesicles as well as the synaptic and neurohemal release. In the present study, I also demonstrated the non-synaptic release of large cored vesicles in the enteric neurons.

As reported in the previous scanning electron microscopic study [5], terminal portion of the enteric nervous system consists of a continuous network of unmyelinated nerve fascicles and does not have a specialized end apparatus facing the effector cells. Therefore, the non-synaptic release coud be expected from these observations. But it was unexpected that the exocytosis also occurred in the sites facing other neuronal processes and Schwann cell's processes. Similar phenomenon was found in the neurosecretory axon terminals of the rat posterior pituitary, where the exocytotic figures of large cored vesicles occurred not only in the sites surrounding blood capillaries but also in the sites facing other neuronal processes (Y. Endo, unpublished data). These facts indicate that the terminal varicosities of neurons may not always release their chemical messengers only in the sites directly facing the target tissues.



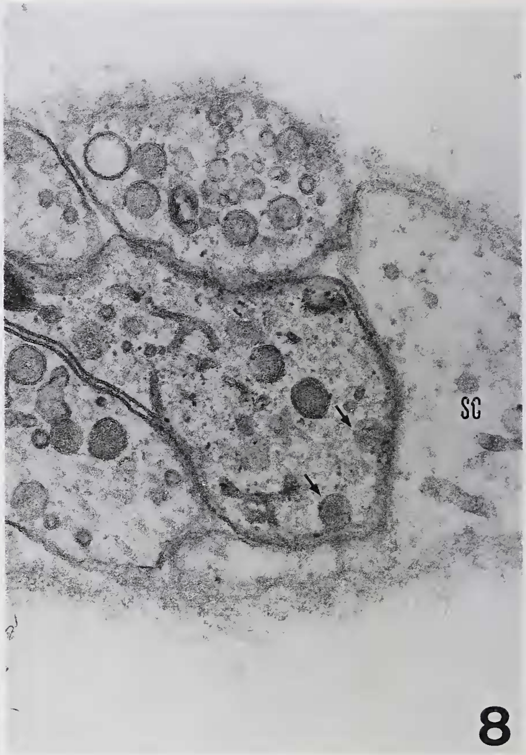


Fig. 7. Exocytotic figure of large cored vesicle (arrow) at the surface of nerve fascicle, distant from smooth muscle cell (S) in the muscularis mucosae. ×69,000.

Fig. 8. Exocytotic figure of large cored vesicle (arrows) at the site facing Schwann cell's process (SC), in the lamina propria mucosae. ×47,000.

ACKNOWLEDGMENTS

The author thanks Prof. S. Kobayashi (Department of Anatomy, Yamanashi Medical College) and Prof. Y. Waku (Department of Applied Biology, Kyoto Institute of Technology) for their encouragements and valuable discussion. This work was supported by a Grant-in-Aid for scientific research from the Ministry of Education, Science and Culture of Japan (No. 62770012).

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ZOOLOGICAL SCIENCE 5: 973–978 (1988) © 1988 Zoological Society of Japan

Fibrillar System and Possible Control Mechanism for the Cycle of Contraction and Elongation of *Spirostomum ambiguum*

Hideki Ishida[1], Yoshinobu Shigenaka[2] and Masako Imada

Laboratories of Cell Biology, Faculty of Integrated Arts and
Sciences, Hiroshima University, Hiroshima 730, Japan

ABSTRACT—A large heterotrichous ciliate, *Spirostomum ambiguum*, shows the characteristic twisting contraction when stimulated by certain mechanical or chemical factors. This contraction is attributed to a special contractile fibrillar system which is termed myoneme, while the subsequent elongation after the contraction occurred might be induced by another type of fibrillar system, longitudinal microtubular sheets (LMSs). The myoneme is composed of a great number of fine filaments which are heavily packed and lie in parallel with each other. The diameter of individual filament varies from 3 to 5 nm in the elongated state but raises up to 7 to 9 nm in the contracted state. Every nonciliated basal body has a rootlet-like structure, which is attached to the myoneme not only in the contracted state but also in the elongated state. If the myoneme and LMSs have antagonistic functions to each other, the force of each fibrillar system is assumed to be transmitted by these rootlet-like structures. On the other hand, anterior fiber sheet was found to be attached to the LMS in the extended state but detached from that in the contracted state of organism. From these observations, the switching or control mechanism was proposed and discussed for the cycle of contraction and elongation of the organism.

INTRODUCTION

Among a number of heterotrichous ciliates, there are some typical contractile ones such as *Condylostoma*, *Spirostomum* and *Stentor*. However, *Spirostomum ambiguum* is the only one showing its own characteristic twisting contraction [1–3]. Although the contraction of the cell body of these organisms is easily induced by various kinds of stimuli this is believed to be caused directly by a special contractile fibrillar system which is termed myoneme [1–6] or M-band [7]. On the other hand, the subsequent elongation of the organism after the contraction occurred has been proposed to be attributed mainly to another fibrillar system, longitudinal microtubular sheets (LMS) [2, 3].

Regarding to the twisting contraction mechanism of *Spirostomum*, Yogosawa-Ohara *et al.* [3] have proposed the following sequence: (1) Shortening along the long axis occurs due to the activity (probably sliding) of myonemal filaments, (2) at the moment when the body length decreases all of the neighboring LMSs slide with each other possibly involving the anterior fiber sheet, and (3) the curving of each LMS is derived from the sliding between the LMS and the sasa structure (SS) by means of obliquely arranged projections on the SS which is a bamboo leaf-like structure attached to the proximal region of every LMS. Coordination of these phenomena might result in the twisting contraction of the organism.

However, the ultrastructural details have not thoroughly been studied especially with respect to the switching mechanism of contraction and elongation of *Spirostomum*. Therefore, the present study aimed to examine the details of myoneme, longitudinal microtubular sheets and their associated structures for elucidating the structural changes between two states of contraction and elongation. As the results, a possible mechanism controlling the contraction-elongation cycle has been proposed here.

Accepted January 28, 1988
Received January 6, 1988

[1] Present address: Biological Institute, Shimane University, Matsue 690, Japan.

[2] To whom reprint requests should be forwarded.

MATERIALS AND METHODS

Organisms

Live samples of *Spirostomum ambiguum* Ehrenberg were originally collected and kindly supplied by Dr. T. Suzaki from the Research School of Biological Sciences, the Australian National University, Canberra, A. C. T. 2601, Australia. They were cultured in our laboratory using 0.01% Knop solution as culture medium containing some boiled wheat grains and small quantity of 0.5% hay infusion at about 24°C. Other special food sources were not supplied to the medium. Subculturing was carried out at a regular interval of about 3 weeks.

Light Microscopy

Light micrographs were obtained from the living organism in both states of contraction and elongation by using a differential interference microscope (Olympus, BH). To cause the contraction, mechanical stimuli were applied to the organisms by means of tapping the specimen-loaded glass slide or electrical stimuli (35 V, DC) were given directly to the organism. In case of electrical stimulation, the organism was placed at the center between the two platinum electrodes (5 mm in distance).

Electron Microscopy

Prior to the fixation, living organisms were rinsed twice to clean them up with fresh culture medium, 0.01% Knop solution. Preparations in the elongated state were prepared by treating the organisms primarily with the relaxation medium containing 10 mM EGTA, 3 mM $MgSO_4$ and 10 mM phosphate buffer (pH 6.8) for 10 min just before the fixation. The other preparations in the contracted state were obtained by stimulating them in the fresh culture medium (0.01% Knop solution) by means of tapping the tube containing the organisms.

The pre-fixation was performed in 1% glutaraldehyde with 33 mM phosphate buffer (pH 6.8) for 30 min at 0°C. The fixed samples were rinsed with 33 mM phosphate buffer (pH 6.8). Following that, they were post-fixed in 1% OsO_4 with 33 mM phosphate buffer (pH 6.8) for 30 min at 0°C. After the double fixation, the samples were dehydrated with a graded ethanol series and embedded into Spurr's low viscosity embedding medium [8].

Ultrathin sections were produced with a Porter-Blum ultramicrotome (MT-1) equipped with glass knives. The sections were stained with 3% aqueous uranyl acetate for 7 min and Reynolds' lead citrate stain [9] for 3 min at room temperature. Observations were carried out under a transmission electron microscope (JEOL, JEM-100S) at the accelerating voltage of 80 kV.

RESULTS

A large heterotrichous ciliate, *Spirostomum ambiguum*, shows the characteristic contraction when applied by chemical, mechanical or electrical stimuli [1–5, 10]. Figure 1 shows the two states of the same free-swimming organism; Figure 1a is in the elongated stage and Figure 1b in the electrical-

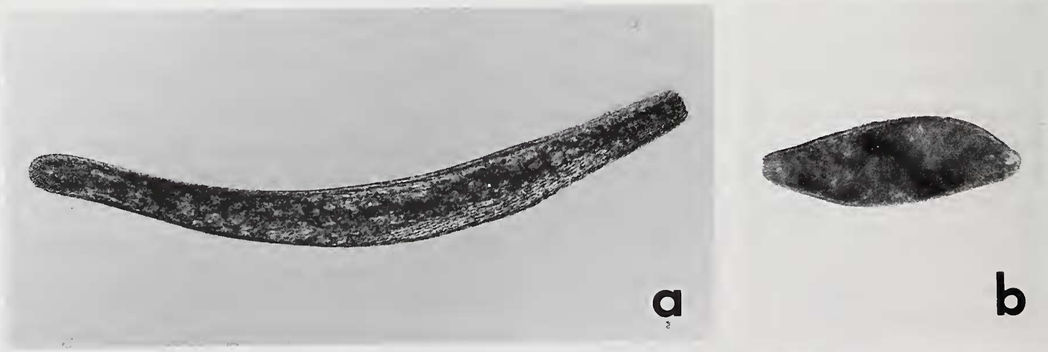

Fig. 1. Light micrographs of the same living *Spirostomum ambiguum* in the extended (Fig. 1a) and the contracted (Fig. 1b) states (×920).

ly stimulated and contracted state.

When observed under an electron microscope, the cortical region of organism is characterized by three typical types of fibrillar systems and their associated structures; sub-pellicular microtubules, longitudinal microtubular sheets and myonemes (Fig. 2a). The myoneme is located at the transitional plane between ectoplasm and endoplasm and featured by being surrounded by various sizes of vacuoles. Every myoneme is composed of a great number of filaments which are heavily packed and lie in parallel with each other inside the myoneme. The diameter of individual filament varies from 3 to 5 nm in elongated state of the organism (Fig. 3a), but raises up to 7 to 9 nm in the contracted state (Fig. 3b).

As a whole, the bundles of myonemal filaments demonstrate a mesh-like distribution throughout the cell body just beneath the arrays of longitudinal microtubular sheets as described by Yogosawa-Ohara and Shigenaka [2]. In addition to that, the present study revealed that major bundles of the myonemal meshes ran in parallel with the body axis in the elongated state of organism but altered their axes diagonally in the contracted state. In the contracted state, on the other hand, the myonemal

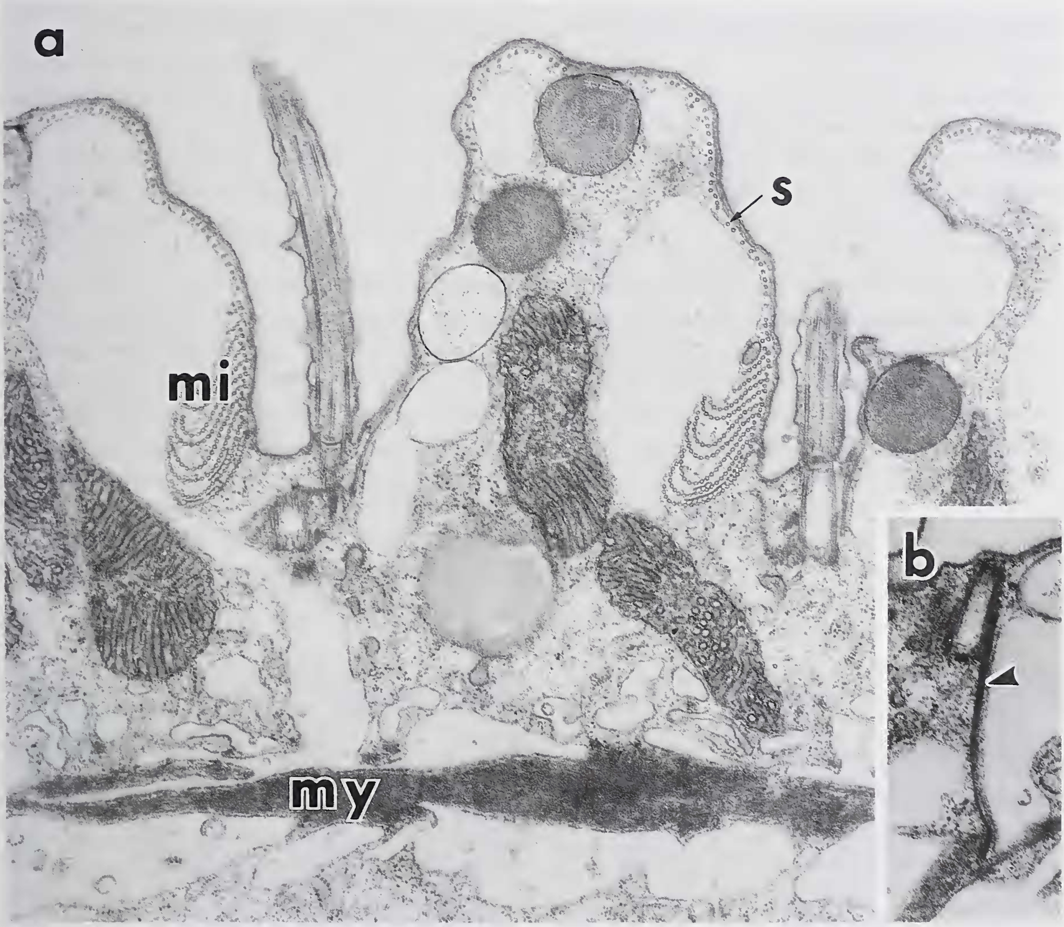

Fig. 2. Electron micrographs of vertical sections through the area of cell cortex. Microtubular sheets (mi) and myoneme (my) lie just under the cell surface (Fig. 2a). An inserted electron micrograph (Fig. 2b) is of the rootlet-like structure in the contracted state, in which a nonciliated posterior basal body was found to be connected with the myoneme by the rootlet-like structure (arrowhead). Just under the cell membrane, sub-pellicular microtubules could be seen (s). (×24,500).